

SERS active colloidal nanoparticles for the detection of small blood biomarkers using aptamers

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ABSTRACT

Functionalized colloidal nanoparticles for SERS serve as a promising multifunctional assay component for blood biomarker detection. Proper design of these nanoproboscopes through conjugation to spectral tags, protective polymers, and sensing ligands can provide experimental control over the sensitivity, range, reproducibility, particle stability, and integration with biorecognition assays. Additionally, the optical properties and degree of electromagnetic SERS signal enhancement can be altered and monitored through tuning the nanoparticle shape, size, material and the colloid's local surface plasmon resonance (LSPR). Aptamers, synthetic affinity ligands derived from nucleic acids, provide a number of advantages for biorecognition of small molecules and toxins with low immunogenicity. DNA aptamers are simpler and more economical to produce at large scale, are capable of greater specificity and affinity than antibodies, are easily tailored to specific functional groups, can be used to tune inter-particle distance and shift the LSPR, and their intrinsic negative charge can be utilized for additional particle stability^{1,2}. Herein, a “turn-off” competitive binding assay platform involving two different plasmonic nanoparticles for the detection of the toxin bisphenol A (BPA) using SERS is presented. A derivative of the toxin is immobilized onto a silver coated magnetic nanoparticle (Ag@MNP), and a second solid silver nanoparticle (AgNP) is functionalized with the BPA aptamer and a Raman reporter molecule (RRM). The capture (Ag@MNP) and probe (AgNP) particles are mixed and the aptamer binding interaction draws the nanoparticles closer together, forming an assembly that results in an increased SERS signal intensity. This aptamer mediated assembly of the two nanoparticles results in a 100x enhancement of the SERS signal intensity from the RRM. These pre-bound aptamer/nanoparticle conjugates were then exposed to picomolar levels of BPA in free solution and the competitive binding event was monitored by the decrease in SERS intensity.

Keywords: Surface enhanced Raman spectroscopy (SERS), aptamer, plasmonic nanoparticles, competitive binding assay, molecular diagnostics

1. INTRODUCTION

Molecularly mediated surface enhanced Raman spectroscopy (SERS) can be a desirable approach for ultrasensitive blood biomarker detection due to its high sensitivity and capability for multiplexing. SERS analysis of DNA-directed nanoparticle assembly using functionalized colloidal nanoparticles has been used extensively as a method to measure and control the degree of enhancement of Raman scattering from a Raman active dye in response to a molecular binding event³. SERS assays in both “turn-on” and “turn-off” formats have been developed using oligonucleotides to detect DNA fragments⁴, as well as with aptamers for the detection of biomarker proteins⁵. In this work we present a “SERS off” competitive binding assay for the detection of BPA: a small molecule toxic to the human endocrine system commonly found in food and beverage packaging.

A schematic representation of the two nanoproboscopes developed for this assay is shown in Figure 1. The ‘target’ silver nanoparticle is functionalized with a mixed self-assembled monolayer consisting of the Raman reporter molecule malachite green isothiocyanate (MGICT) and BPA aptamers. These particles are designed to bind to a version of the toxin bisphenol A diglycidyl ether (BADGE), immobilized onto a second silver coated magnetic ‘probe’ nanoparticle, where binding affinities have been determined previously by Marks et al⁶. The assembled nanoparticle network provides a localized ‘hot spot’ of electric field enhancement around the RRM, and therefore an increase in the number of scattering

events experienced by the Raman reporter that is observable in the SERS spectral intensity. Upon introduction of free BPA in its native form, the bound AgNPs are competitively freed; reversing the nanoparticle assembly and causing the SERS signal to “turn-off” and decrease in response to the competitive binding event.

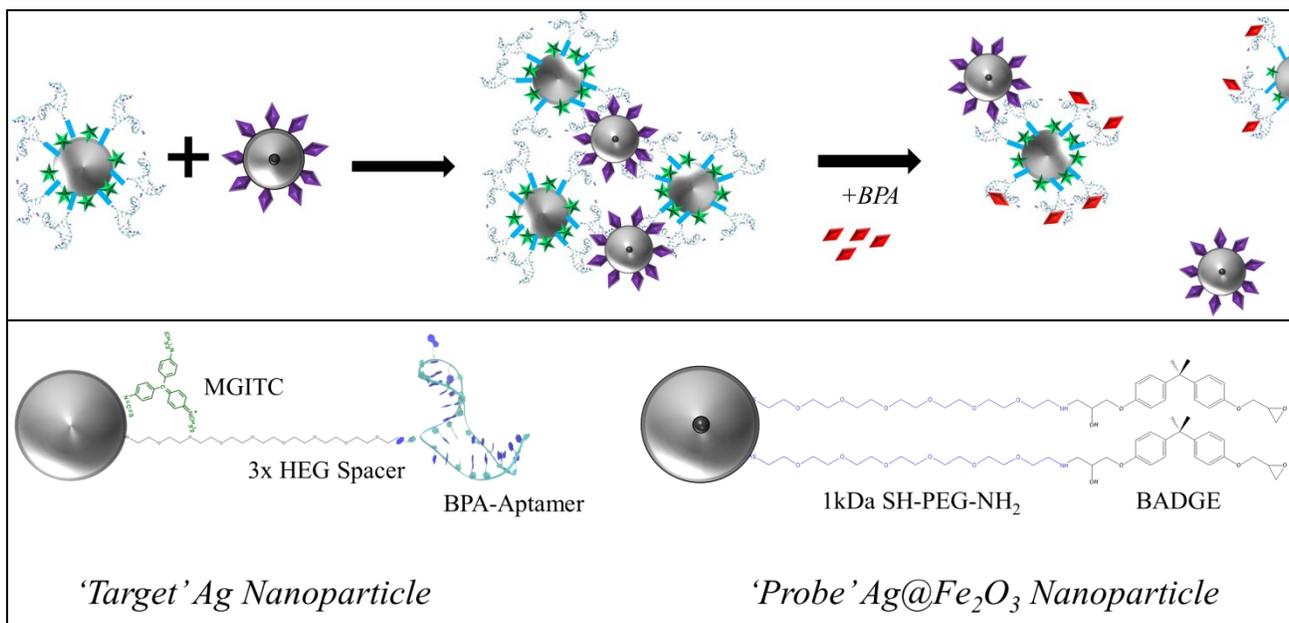


Figure 1. Top: Schematic illustration of the aptamer mediated ‘turn-off’ SERS competitive binding assay. Bottom: Target and probe nanoparticle conjugate components.

2. MATERIALS & METHODS

2.1 Materials and Instrumentation

The modified BPA aptamer⁷: 5'- [ThiSS][HEG]₃ CCG CCG TTG GTG TGG TGG GCC TAG GGC CGG CGG CGC ACA GCT GTT ATA GAC GTC TCC AGC-3' was synthesized by Eurofins Genomics (Germany). Hetero-bifunctional PEG linker (NH₂-PEG-SH, 1kDa) was purchased from NanoCS (USA). Malachite green isothiocyanate reporter dye was purchased from Invitrogen (UK). All other reagents were obtained from Sigma Aldrich (USA/UK).

A Varian Cary 300Bio UV-Visible Spectrophotometer was used with a scan range of 200-800 nm for extinction measurements. The zeta potential and hydrodynamic diameters of the nanoparticles were measured on a Zetasizer Nano ZS90 (Malvern, U.K.). SERS measurements were collected using a WiTec Raman instrument equipped with a 532 nm laser.

2.2 Colloid Synthesis

Silver colloid (AgNP) was synthesized using the reduction method as reported by Leopold and Lendl⁸. Hydroxylamine hydrochloride (1 ml, 150 mM) was added to 89mL of NaOH (3.33 mM) under vigorous stirring. Silver nitrate (AgNO₃) solution (10 mM) was added drop-wise and stirred for 15 min at room temperature. DLS measurements revealed an average particle diameter of ~45 nm and the stock particle concentration was determined to be 145 pM according to Beer’s Law using an extinction coefficient of $2.87 \times 10^{10} \text{ M}^{-1} \text{ cm}^{-1}$ at 404 nm.

Silver-coated magnetic nanoparticles (Ag@MNP) were prepared using a co-precipitation method for the synthesis of the particle core and glucose reduction to coat with silver as described by Kumar et al⁹ and Mandal et al¹⁰ respectively. Briefly, a stock solution of maghemite (γ -Fe₂O₃) nanoparticles was prepared by adding 25 mL of an acidified iron salt solution (0.4 M Fe²⁺, 0.8 M Fe³⁺, 1 M HCl) drop-wise to 250 mL of 1.5 M NaOH at 50°C under vigorous non-magnetic stirring. After 20 minutes the particles were allowed to cool, washed twice with DI water and once with 0.1M HNO₃. An additional 125 mL of HNO₃ is then added to the solution, stirred an additional 40 minutes at 95°C, and resuspended in distilled water.

To coat with silver, 1 mL of these stock MNPs was mixed with 4 mL of 0.35 M glucose and 1.5 mL of 60 μM AgNO_3 . The solution was sonicated for 10 minutes then heated to 90°C for 90 minutes. Finally, the particles were centrifuged three times to wash, and redispersed in 6 mL of 5 mM sodium citrate for a particle diameter of ~ 31 nm and final concentration of 608 pM as previously reported¹¹.

2.3 Target and Probe Nanoparticle Conjugation

Aptamer/RRM AgNP Target Synthesis

Prior to immobilization, the BPA aptamers were suspended in a 60 mM phosphate buffer (PBS, pH 8.5) and treated with 15 mg of dithiothreitol (DTT) to reduce their disulfide bonds. After one hour the aptamers were HPLC purified to remove residual DTT, and then heated to 90°C for 5 minutes to fold the aptamers into their tertiary structure for optimal binding.

Target AgNPs were functionalized with the aptamer in accordance with the method developed by Zhang et al¹². The aptamer (7 μM) was added to 1 mL of silver colloid (145 pM) at a molar ratio of 2000:1 and left for 1 hour. Three 20 μL aliquots of 250 mM citrate HCl buffer (pH 2.9) were then added 5 minutes apart, and the sample was left an additional 30 minutes before centrifuging and resuspending the aptamer functionalized particles in 1 mL of 0.3 M PBS (pH 7.4). The Raman reporter molecule, malachite green isothiocyanate (MGICT, 10 μM in methanol), was then added at a 500:1 ratio and left to react with the aptamer AgNPs under sonication for 1 hour, which were then centrifuged and stored in a 0.1 M PBS buffer (pH 7.4).

Analyte Ag@MNP Probe Synthesis

To synthesize 1 mL of BADGE functionalized magnetic nanoprobe, the SH-PEG-NH₂ linker was added drop-wise to an excess of the analyte BADGE in 0.3 M PBS buffer (pH 8.5) and left overnight to allow conjugation between their terminal amine and epoxide groups respectfully. The conjugates were then treated with aminoethanol to open unbound epoxide rings and prevent non-specific binding of BADGE to the RRM's amine groups. These SH-PEG-BADGE conjugates were then added at a 5000:1 ratio to 1 mL of silver coated magnetic nanoparticles (diluted to 200 pM). After one hour three 20 μL aliquots of 250 mM citrate HCl buffer were again added 5 minutes apart, and the sample was left an additional 30 minutes before centrifuging and resuspending the BADGE functionalized particles in 1 mL of 0.1 M PBS (pH 7.4).

2.4 SERS analysis

Equal volumes containing 30 pM of each of the target and probe nanoparticles were left overnight to allow complete binding between the immobilized BPA aptamers and BADGE. The assembled nanoparticle clusters were collected using a neodymium magnet held at the side of the glass vial for 30 minutes, the supernatant containing any unbound AgNPs was removed, and the assembled nanoparticles were resuspended in 0.1 M PBS (pH 7.4). All SERS measurements were collected with the target AgNP concentration held constant at 7.5 pM in a total volume of 30 μL of 0.1 M PBS (pH 7.4) with an integration time of 10 seconds (1 s exposure). For competitive binding analysis 15 μL of the assembled target/probe NPs was mixed with 15 μL of 1 μM BPA in 0.1 M PBS (pH 7.4) and SERS measurements were taken every 30 seconds.

3. RESULTS AND DISCUSSION

The target and probe nanoparticles were functionalized as described and yielded average zeta potential measurements of -39.7 and -34.6 mV respectively, indicating that the PEG spacer and the HEG modified aptamer provide adequate particle stabilization in the 0.1 M PBS binding buffer. The intrinsic SERS signal of a stock solution of the assembled nanoparticles and its response to the presence of competing BPA was then analyzed. As shown in Figure 2, the SERS signal of the reporter molecule MGICT increases by two orders of magnitude as it experiences an enhancement in the electric field intensity of its immediate environment due to the aptamer-binding induced particle aggregation. When free BPA is added to the conjugate solution, it is observed that the SERS intensity of MGICT's 1175 cm^{-1} peak (aromatic C-H bending vibrational mode¹³) decreases to a steady state over the course of ~ 5 min as competitive binding occurs (Fig. 2, inset). That is to say, the BPA aptamer immobilized on the target AgNPs releases from the BADGE on the MNPs in order to more favorably bind to free BPA, and the released particles return to colloidal suspension causing a decrease in the solution SERS signal as hypothesized.

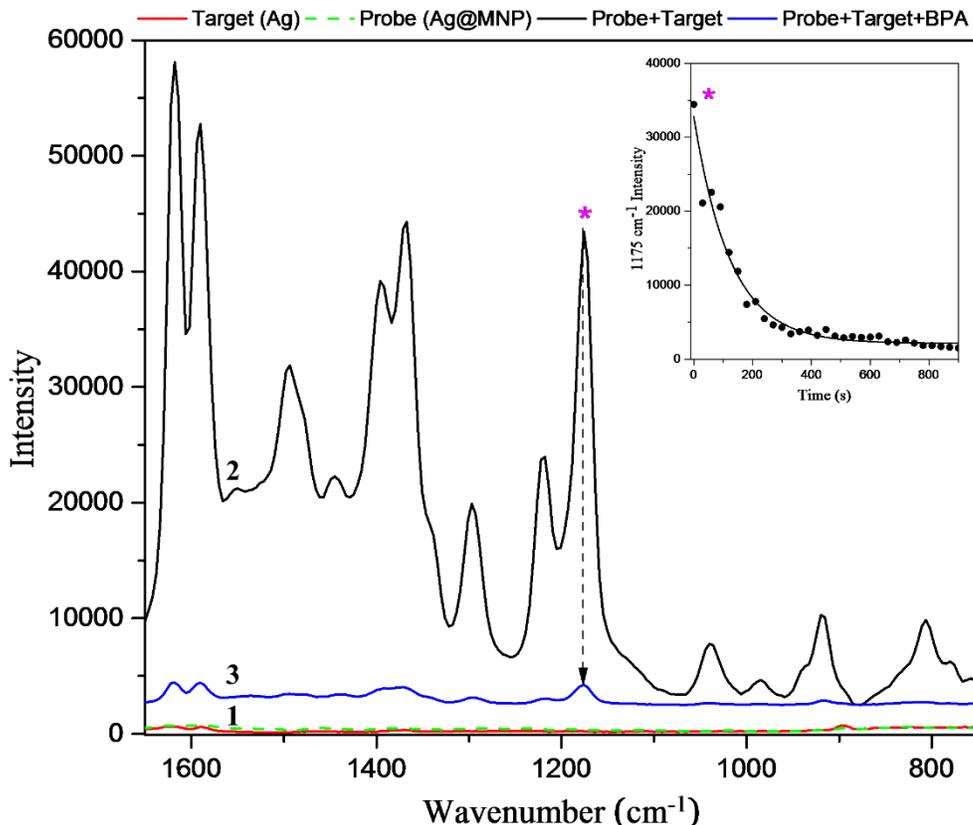


Figure 2. SERS spectra of the target and probe nanoparticles: (1) individually, (2) mixed overnight to allow complete NP assembly via aptamer/BADGE binding, and (3) assembled NPs after exposure to 15 pmol of the competing analyte. Inset: SERS monitoring of the competitive binding event over 15min.

4. CONCLUSION

In summary, we have described a “turn-off” SERS assay platform methodology for the detection of small toxins utilizing aptamer-mediated assembly of SERS active colloidal nanoparticles. The SERS assay was able to monitor a five minute competitive binding event initiated by the presence of 15 pmols bisphenol A in free solution. However, detailed characterization of the dose-response relationship and coefficients of variation of the system will assist in further development of this molecular recognition platform. Additionally, this approach has the potential to be translated to any aptamer/antigen pair and provides the added benefit of magnetic manipulation of the nanoparticle sensing network.

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